

Supplemental Amendment for application 10/037, 718 Applicants MCGINNIS ET AL. 3  
August 2, 2006 submitted by fax to 571-273-8300 Total of 23 pages

The applicants hereby request an amendment to the specification of the application. The applicants believe that this amendment is in accordance with US practice including 37 CFR 1.121.

***In the Specification***

Please add the following three new paragraphs after paragraph [0249 2] and before paragraph [0250]:

[0249.3] Other versions of oligonucleotide technology use oligonucleotides as PCR primers and generate a physico-chemical signal. Saiki, et. al.<sup>11</sup> describes using oligonucleotides as PCR primers for genotyping. A method is described by which one can simultaneously screen a sample for all known allelic variants at an amplified locus. The target segment of DNA sample to be tested is PCR amplified with biotinylated primers and then hybridized to a nylon membrane containing immobilized sequence-specific oligonucleotide probes. Hybridization is detected nonradioactively by the binding of streptavidin-horseradish peroxidase to the biotinylated DNA, followed by a simple colorimetric reaction. PCR amplification of genomic sequences was performed as follows. DNA (0.1-0.5 µg) was amplified in 100 µl containing 50 mM KCl, 10mM Tris HCl (pH 8.4), 1.5 mM MgCl<sub>2</sub>, 10 µg gelatin, 200 µM each of dATP, dCTP, dGTP, and dTTP, 0.2 µM of each biotinylated amplification primer and 2.5 units of Taq DNA polymerase.

[249.4] Wu, et. al.<sup>11</sup> describes using oligonucleotides as PCR primers for genotyping. A rapid nonradioactive approach to diagnosing sickle cell anemia is described. Two allele-specific primers, one specific for sickle cell anemia and one specific for the normal allele, together with another primer complementary to both alleles were used in the polymerase chain reaction with genomic DNA templates. (Sickle cell anemia is a genetic disease caused by a single base-pair mutation.) PCR primers were used at a concentration of 0.12 µM; reactions were performed in a volume of 50 µl. And PCR products were analyzed by gel electrophoresis, staining of the gel with ethidium bromide (and destaining with water). The stained gel was then photographed by ultraviolet trans-illumination. An alternative strategy wherein each allele-specific primer is labeled with a fluorescent group (such as fluorescein or tetramethyl rhodamine) is also described. The third PCR primer is labeled with biotin. The PCR products are then captured on streptavidin-agarose and the presence of amplified sequence is detected with fluorescence.

[249.5] Nickerson, et. al.<sup>11</sup> describes a strategy for DNA typing that is automated and nonisotopic. The strategy uses amplification of target DNA segments by PCR and the discrimination of allelic variants by a colorimetric oligonucleotide ligation assay (OLA). This strategy or "PCR/OLA procedure" uses oligonucleotides as PCR primers. DNA amplification was carried out using PCR reagents assembled by robotic workstation. The assembled PCR reagents consisted of 5 µl that contained 0.5 µM of amplification primers and other constituents.